PAM Application Notes (2008) 1: 25 - 26

Estimating epidermal UV-A and UV-B screening in leaves with a XE-PAM Fluorometer

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Abstract

This communication recapitulates step-by-step how epidermal transmittance for ultraviolet (UV) radiation can be determined by chlorophyll (Chl) fluorescence measurements using a XE-PAM fluorometer (*cf.* Bilger *et al.* 1997).

Introduction

Absorption of UV radiation by epidermal phenolics (flavonoids and hydroxycinnamic acids) represents the first line of plant defense against UV damage. Therefore, information on epidermal UV transmittance is a prerequisite to understand plant growth under natural radiation conditions. Bilger *et al*. (1997) have introduced a fluorometric approach to determine UV screening in leaves. Particular benefits of the method are:

- **Flexibility**. Epidermal transmittance can be determined at various wavelengths including the short-wavelength UV-B radiation. In principle, the method is not limited to the UV but can be extended to the visible range to determine, for example, *in vivo* screening by anthocyanins.
- **Simplicity**. Sample preparation and measurements are so easy that large sample numbers can be readily examined.

Here, the XE-PAM fluorometer was employed to determine epidermal UV transmittance in barley and orchard grass leaves.

Material and methods

Plants

Three-weeks old plants of the barley (*Hordeum vulgare* L.) Ant 287 mutant which is defective in flavonoid biosynthesis (Reuber *et al*. 1996), and its wild-type variety (Hege 550/75) were grown indoors. Orchard grass (*Dactylis glomerata* L.) grew outdoors in a sun-exposed habitat. Prior to fluorometry, leaves were acclimated for at least 1 hour to dim light (3 µmol·m^{-2}·s^{-1}).

Instrumentation

Fluorescence was measured by a XE-PAM fluorometer controlled by the WinControl V2.08 software *via* the PAM data-acquisition system (PDA-100) Using double-sided adhesive tape, samples were attached with their lower leaf side to a UV leaf holder (Walz). The holder was placed in the ED-101US/M optical unit in which excitation radiation, guided by a quartz rod, reached the sample at an angle of 45°. Fluorescence was detected at right angles to the excitation beam. Fo level fluorescence was elicited by flashes delivered at 2 Hz from a Xe lamp. The following filter combinations were used:

Excitation	
UV-B	DUG11[a] & UG11[a] sandwich, GG19[a]
UV-A	DUG11 & UG11 sandwich, BG39[a]
Blue-Green	BG 39, UV-blocking filter[b], 2% PH [c]
Emission	
Red	R65[b] & RG645[a] sandwich, UV-blocking filter

Filters are engineered by Walz to fit into the filter holder of the XE-PAM measuring flash lamp and photodiode detector, respectively. [a]Schott, Mainz, Germany. [b]Balzers, Liechtenstein. [c]Pinhole transmitting 2% of excitation radiation.

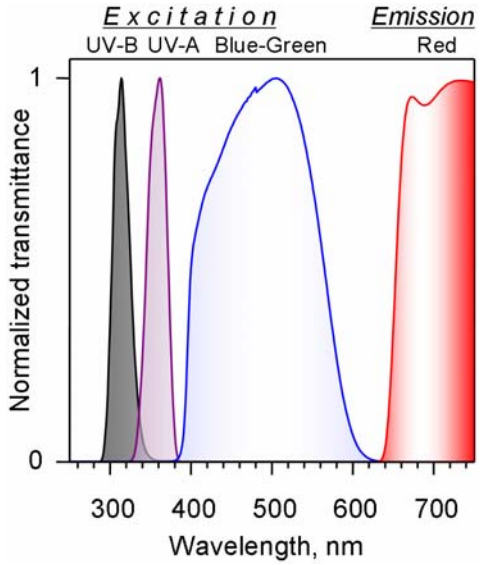

Fig. 1. Normalized transmittance of excitation and emission filters used to determine *in vivo* epidermal transmittance.

Measuring Procedure

Rationale

Chl fluorescence can be excited by visible and UV radiation. In the case of an intact leaf, epidermal UV screening interferes with UV excitation of Chl. Hence, the level of UV-excited Chl fluorescence is related to epidermal UV transmittance, provided the quantum yield of Chl fluorescence is constant. In the dark-acclimated state, leaves emit fluorescence with the constant Fo yield. Therefore, it was assured that Fo fluorescence was recorded by (1)

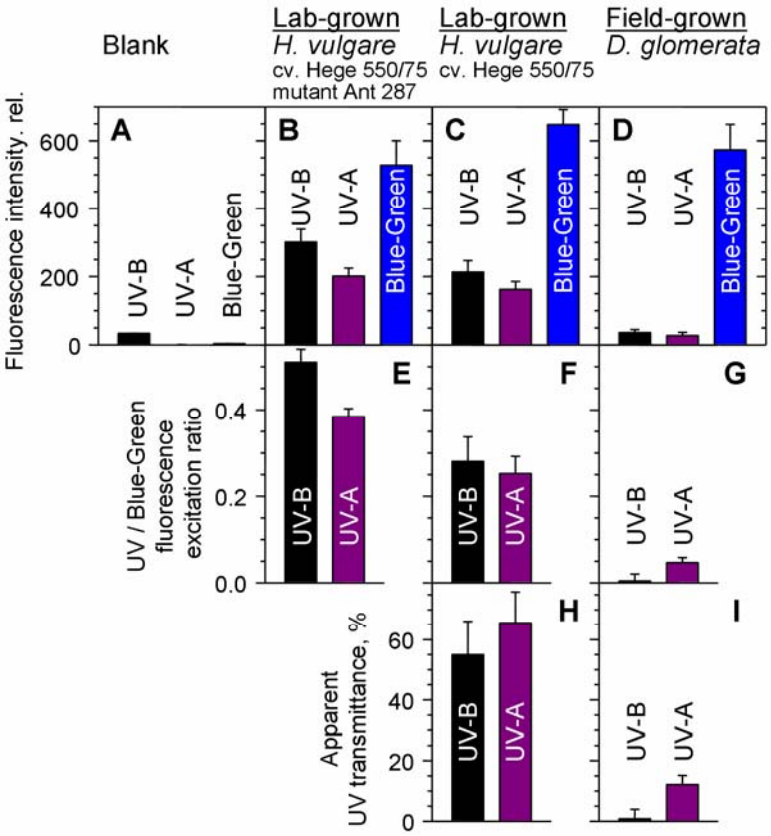

Fig. 2. **Determination of epidermal UV transmittance.**

A to **D**: absolute signal levels recorded with a blank UV leaf holder (A), barley Ant 287 mutant leaves (B), barley wild type leaves (C) and orchard grass leaves (D), respectively. Excitation was in the UV-B, UV-A and Blue-Green spectral range (see Fig. 1).

E to **G**: UV/Blue-Green fluorescence excitation ratios (FER) calculated from absolute signal levels. Prior to calculation of FER, the signals obtained with the blank (A) were subtracted from corresponding signal levels in B – D.

H to **I**: Apparent transmittance for UV-B and UV-A radiation in lab grown wild type leaves of barley and in field-grown leaves of orchard grass.

Data correspond to means of 14 measurements with different leaves (error bars show standard deviations).

plant pre-acclimation to dim light and (2) by low integrated excitation light intensities as provided by the XE-PAM fluorometer (*cf.* Schreiber *et al.* 1993).

Three steps to epidermal UV transmittance

(1) Leaf fluorescence was excited by UV-B, UV-A and (reference) blue-green radiation (*cf.* Fig. 1; Fig. 2B-D). For the three excitation conditions, background signals were assessed using a blank UV leaf holder (Fig. 2A). Background signals were always subtracted from the corresponding leaf signals prior to further processing of data.

(2) Different from UV radiation, blue-green radiation is transmitted by the epidermis of green leaves. The UV/blue-green fluorescence excitation ratio (FER; Fig. 2E-G) cancels out all those leaf optical properties which affect UV and blue-green excitation of fluorescence in a similar way. Therefore, epidermal UV screening is the main leaf optical factor determining the FER. In addition, however, the FER also depends on the ratio of UV and blue-green excitation intensities.

(3) The epidermis of the ANT 287 barley mutant can be considered as practically UV transparent, because the mutant leaves lack most flavonoids (Reuber *et al.* 1996), and, generally, hydroxycinnamic acids provide insignificant UV screening in barley leaves (Kolb and Pfündel 2005). Therefore, the FER obtained with the barley mutant (Fig. 2E) represents unscreened leaf mesophyll and, hence, relating the FER from wild-type leaves to the mutant FER cancels out unbalanced excitation intensities. In this way, data of epidermal transmittance for UV radiation are obtained (Fig. 2 H and I).

Consistent with the well-known induction of UV screening by UV stress, in indoor-grown leaves (i. e., in the absence of natural UV radiation), epidermal transmittance was rather high (> 50%), but in field-grown leaves, UV-B transmittance dropped to 1% (Fig. 2I).

Here, we used intact leaves from the flavonoid-deficient barley mutant as mesophyll reference. For other leaf types, preparation of authentic mesophyll references by stripping off the epidermis may be necessary. In many leaves it is difficult to remove the epidermis and a blue plastic fluorescence standard (Walz), which resembles the fluorescence excitation properties of *Vicia faba* mesophyll, might be used to substitute for the actually varying fluorescence properties of mesophyll in different leaf types.

References

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